

# Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs<sup>1</sup>

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**ABSTRACT:** Lysozyme is a 1,4- $\beta$ -N-acetylmuramidase that has antimicrobial properties. The objective of this experiment was to determine if lysozyme in nursery diets improved growth performance and gastrointestinal health of pigs weaned from the sow at 24 d of age. Two replicates of 96 pigs (192 total; 96 males, 96 females) were weaned from the sow at 24 d of age, blocked by BW and gender, and then assigned to 1 of 24 pens (4 pigs/pen). Each block was randomly assigned 1 of 3 dietary treatments for 28 d: control (two 14-d phases), control + antibiotics (carbadox/copper sulfate), or control + lysozyme (100 mg/kg diet). Pigs were weighed and blood sampled on d 0, 14, and 28 of treatment. Blood was analyzed for plasma urea nitrogen (PUN) and IgA. At 28 d, pigs were killed, and samples of jejunum and ileum were collected and fixed for intestinal morphology measurements. An additional jejunum sample was taken from the 12 pigs with the median BW per treatment to determine transepithelial electrical resistance (TER). Pigs consuming antibiot-

ics or lysozyme grew at a faster rate than control pigs ( $0.433 \pm 0.009$  and  $0.421 \pm 0.008$  vs.  $0.398 \pm 0.008$  kg/d, respectively;  $P < 0.03$ ), which resulted in heavier ending BW ( $20.00 \pm 0.31$ ,  $19.8 \pm 0.29$ , and  $18.83 \pm 0.32$  kg, respectively;  $P < 0.03$ ). Feed intake was not different ( $P > 0.48$ ), but G:F was improved in pigs consuming antibiotics or lysozyme ( $0.756 \pm 0.014$ ,  $0.750 \pm 0.021$ , and  $0.695 \pm 0.019$  kg/kg;  $P < 0.05$ ). Immunoglobulin A ( $P < 0.03$ ) and PUN ( $P < 0.01$ ) increased during the experiment, regardless of dietary treatment ( $P > 0.48$ ). Dietary treatment did not affect TER ( $P > 0.37$ ), but gilts had lower TER compared with barrows ( $P < 0.04$ ). No differences in villi height or crypt depth were observed in the ileum ( $P > 0.53$ ). However, jejunum villi height was increased and crypt depth was decreased in pigs consuming antibiotics or lysozyme ( $P < 0.001$ ), resulting in an increased villi height: crypt depth of 72% ( $P < 0.001$ ). Thus, we concluded that lysozyme is a suitable alternative to carbadox/copper sulfate diets fed to pigs weaned from the sow at 24 d of age.

**Key words:** antimicrobial, growth, intestine, lysozyme, swine

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## INTRODUCTION

Lysozyme is a 1,4- $\beta$ -N-acetylmuramidase that enzymatically cleaves a glycosidic linkage in the peptidoglycan component of bacterial cell walls, which results in the loss of cellular membrane integrity and cell death (Ellison and Giehl, 1991). In addition, hydrolysis products are capable of enhancing IgA secre-

tion, macrophage activation, and rapid clearance of bacterial pathogens (Kawano et al., 1981; Clarke et al., 2010; Silhavy et al., 2010). These data indicate that lysozyme may prove to be a viable alternative to antibiotics in swine feed to ameliorate the effects of a disease challenge.

Until recently, the literature pertaining to lysozyme as a feed additive was limited to studies using transgenic goat milk or transgenic rice to produce and deliver the enzyme. These studies have shown changes in metabolite profiles (Brundige et al., 2010), intestinal microflora (Maga et al., 2006), and intestinal morphology (Brundige et al., 2008) in pigs fed milk from transgenic goats expressing human lysozyme in the mammary gland. In addition, Humphrey et al.

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(2002) reported that diets supplemented with transgenic rice expressing lysozyme had antibiotic-like properties when fed to chicks. Although these reports are encouraging, the delivery of lysozyme from transgenic goat milk or transgenic rice is problematic in a swine production setting. Recently, our laboratories (May et al., 2012) have shown that lysozyme sourced from chicken eggs (Neova Technologies Inc., Abbotsford, BC, Canada) improved growth rate and intestinal morphology and reduced *Campylobacter* shedding in 10-d-old pigs consuming a milk diet. However, it is unknown if lysozyme in conventional nursery diets improves performance similarly to young pigs consuming manufactured liquid diets. Therefore, the objective of this experiment was to determine if lysozyme in nursery diets impacts growth performance and gut health of pigs weaned from the sow at 24 d of age.

## MATERIALS AND METHODS

The experimental protocol was approved by the Animal Care and Use Committee of the U.S. Meat Animal Research Center (USMARC).

### *Animal Care and Dietary Treatment*

Two replicates of 96 pigs (192 total; 96 males, 96 females) were weaned from the sow at 24 d of age to the USMARC experimental animal facility and used in a randomized complete block design. Pigs were blocked by BW and gender and then assigned to 1 of 24 pens (4 pigs/pen). Each block was randomly assigned either to the control diets (two 14-d phases), control diets + antibiotics (carbadox/copper sulfate; 55 mg carbadox and 250 mg Cu/kg, respectively), or control diets + lysozyme (100 mg/kg diet). All diets met or exceeded NRC recommendations for required nutrients (Table 1; NRC, 1998). Pigs were allowed to consume diets ad libitum for 28 d. Feed disappearance was measured on a weekly basis, and pig BW were determined gravimetrically on d 0, 14, and 28 of treatment.

### *Sample Collection and Analytical Procedures*

On d 0, 14, and 28 of treatment, 5 mL of blood was collected into heparinized syringes (20 IU of Li-heparin/mL blood) via jugular venipuncture and placed immediately on ice. After collection, blood samples were centrifuged at  $800 \times g$  for 10 min at 4°C, with plasma collected and frozen at -20°C until further analyses. Plasma was analyzed for IgA and plasma urea N (PUN) concentrations. The IgA concentrations were determined in duplicate by commercial ELISA (Bethyl Laboratories Inc., Montgomery, TX) using porcine IgA as standards.

Plasma was analyzed for urea N in duplicate by using a commercial colorimetric kit (BioAssay Systems, Hayward, CA). The sample mean for pooled plasma PUN was  $5.0 \pm 0.2$  mM and the intra- and interassay CV were 4.2% and 4.6%, respectively. The sample mean for IgA pools was  $8.9 \pm 0.6$  mg/mL, and the intra- and interassay CV were 4.8% and 4.5%, respectively.

Pigs were killed via euthanasia solution (Beuthanasia-D, Shering-Plough Animal Health Corp., Union, NJ) on d 28 of treatment. One 3-cm segment of mid-jejunum and one 3-cm segment of mid-ileum were collected from all pigs for histological analysis as previously described (Oliver et al., 2002). An additional 3-cm segment of jejunum was taken from the 12 pigs with the median BW (equal male and female) per treatment to determine transepithelial electrical resistance (TER). One segment of jejunum and one of ileum were processed, embedded, and stained according to previously described procedures (Luna, 1968). Briefly, freshly cut intestinal sections were rinsed in cold PBS and then fixed in freshly prepared chilled fixative solution (FEA: 10 mL formalin, 70 mL 95% ethanol, 15 mL distilled water, 5 mL acetic acid). Intestinal segments were dehydrated over a 2-d period using increasing concentrations of ethanol and chloroform. Sections were embedded in paraffin, and cross sections were cut with a microtome (American Optical Co., Buffalo, NY) approximately 10  $\mu$ m thick. Sections were stained with hematoxylin and eosin, and morphometric measurements were performed by 1 person (blinded to treatment) using light microscopy with a computer-assisted morphometric system (Bioquant Image Analysis Corp., Nashville, TN). The height and crypt depth of 8 well-oriented villi per sample were measured.

Jejunum mucosal barrier function was accessed via TER according to previously published protocols (Argenzio and Liacos, 1990; Moeser et al., 2007). Briefly, the segment of mid-jejunum was collected from the pig, and the mucosa was stripped from the seromuscular layer in oxygenated Ringer solution. Tissues were then mounted on Ussing chambers and bathed on the serosal and mucosal sides with 10 mL of Ringer solution. The mucosal bathing solution contained 10-mM glucose, which was osmotically balanced on the serosal side with 10-mM mannitol. Bathing solutions were oxygenated and circulated in water-jacketed reservoirs maintained at 37°C. After 15 min, the spontaneous potential difference (PD) was measured via Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through a fluid resistance-corrected voltage clamp through Ag-AgCl electrodes. Tissues were maintained in the short-circuited state, except for brief periods to record the open-circuit PD. Transepithelial electrical resistance was calculated, as previously described (Argenzio and Liacos, 1990), from the PD and short-circuit current.

**Table 1.** Composition and calculated analysis of the dietary treatments<sup>1</sup>

Item	Phase 1 (d 0 to 14)			Phase 2 (d 14 to 28)		
	Control	C + A	C + Lyso	Control	C + A	C + Lyso
Ingredients, %						
Corn	50.8	49.6	50.5	63.3	62.1	63.0
Soybean meal, 465 g/kg	24.3	24.4	24.3	26.5	26.6	26.6
Fish meal	5.0	5.0	5.0	2.5	2.5	2.5
Blood meal	1.3	1.3	1.3	1.3	1.3	1.3
Whey	12.5	12.5	12.5	0.0	0.0	0.0
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate	0.8	0.8	0.8	1.2	1.2	1.2
Limestone	0.8	0.8	0.8	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.4	0.4	0.4
Zinc oxide	0.3	0.3	0.3	0.0	0.0	0.0
Vitamin premix <sup>2</sup>	0.3	0.3	0.3	0.3	0.3	0.3
Mineral premix <sup>3</sup>	0.2	0.2	0.2	0.2	0.2	0.2
Lysine HCl, 980 g/kg	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine, 985 g/kg	0.2	0.2	0.2	0.1	0.1	0.1
L-Threonine, 985 g/kg	0.2	0.2	0.2	0.1	0.1	0.1
Carbadox	0.0	1.0	0.0	0.0	1.0	0.0
Copper sulfate	0.0	0.1	0.0	0.0	0.1	0.0
Lysozyme <sup>4</sup>	0.0	0.0	0.3	0.0	0.0	0.3
Calculated analysis <sup>5</sup>						
ME, MJ/kg	14.4	14.3	14.4	14.4	14.3	14.4
CP, g/kg	210	210	210	209	208	209
TID lysine, <sup>6</sup> g/kg	13.5	13.5	13.5	13	13	13
Ca, g/kg	8.7	8.7	8.7	8.1	8.1	8.1
Available P, g/kg	4.4	4.4	4.4	3.8	3.8	3.8

<sup>1</sup>Expressed on as-fed basis: C + A = control plus antibiotics; C + Lyso = control plus lysozyme.

<sup>2</sup>Provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D<sub>3</sub>, 200 IU; vitamin E, 19 IU; vitamin K, 2.2 mg; thiamine, 2.2 mg; riboflavin, 4.4 mg; niacin, 33 mg; pantothenic acid, 22 mg; vitamin B<sub>12</sub>, 0.028 mg; vitamin B<sub>6</sub>, 2.2 mg; folic acid, 1.35 mg; and biotin, 0.11 mg.

<sup>3</sup>Provided per kilogram of diet: Fe, 78 mg; Cu, 7 mg; Co, 0.80 mg; Zn, 168 mg; Mn, 60 mg; I, 0.79 mg; and Se, 0.13 mg.

<sup>4</sup>Entegaurd, Neova Technologies Inc., Abbotsford, BC, Canada; provides a final concentration of 100 mg lysozyme/kg diet.

<sup>5</sup>Calculated analysis based on standard feed tables (NRC, 1998).

<sup>6</sup>TID = true ileal digestible.

## Statistical Analyses

Data were subjected to ANOVA using the GLM procedure (Minitab Inc., State College, PA). Data were evaluated for the effects of treatment (control, antibiotics, and lysozyme), day, sex, and all appropriate interactions. For responses where no statistically significant sex differences were observed, sex data were combined. Day, treatment, and sex (where appropriate) replication were contrasted using a protected LSD test (Steel et al., 1997). Similar to previous experiments (Oliver and Miles, 2010; May et al., 2012; Oliver et al., 2012), pen of pigs was the experimental unit for evaluation of the effects of diet on feed intake and feed efficiency, and individual pig was the experimental unit for all other statistical procedures. The significance level for all tests was set at  $P < 0.05$ .

## RESULTS

Pigs were weaned at  $23.7 \pm 0.1$  d of age and weighed  $7.8 \pm 0.1$  kg, regardless of dietary treatment ( $P > 0.72$ ). In addition, no gender differences were observed ( $P > 0.21$ ). From d 0 to 14 of treatment, pigs consuming antibiotics grew at a faster rate than pigs consuming both the control and lysozyme diets (Table 2;  $P = 0.036$ ). From d 14 to 28, pigs fed lysozyme or antibiotic in their diets gained at a similar rate ( $P = 0.59$ ), which was greater than pigs consuming the control diets ( $P = 0.017$ ). This resulted in an overall greater ADG from d 0 to 28 for pigs consuming antibiotics or lysozyme compared with control pigs ( $P = 0.024$ ). Because of the increase in ADG, pigs consuming the antibiotic ( $P = 0.022$ ) or lysozyme ( $P = 0.023$ ) diets were heavier on d 28 of the study compared with the control pigs ( $P < 0.03$ ). Pigs consumed  $2.27 \pm 0.06$  kg diet $\times$ pen<sup>-1</sup> $\times$ d<sup>-1</sup> over the course of treatment, regardless of dietary treatment ( $P = 0.48$ ). Because of similar feed consumption rates and increased ADG in

**Table 2.** Performance by pigs weaned at 24 d of age and fed control, control plus antibiotics (C + A), or control plus lysozyme (C + Lyso) diets for 28 d<sup>1</sup>

Variable	Diet		
	Control	C + A	C + Lyso
BW, kg			
d 0	7.73 ± 0.12	7.83 ± 0.13	7.85 ± 0.15
d 14	11.09 ± 0.20 <sup>a</sup>	11.60 ± 0.19 <sup>b</sup>	11.41 ± 0.19 <sup>b</sup>
d 28	18.83 ± 0.32 <sup>a</sup>	20.00 ± 0.31 <sup>b</sup>	19.80 ± 0.29 <sup>b</sup>
ADG, kg/d			
d 0 to 14	0.243 ± 0.009 <sup>a</sup>	0.271 ± 0.010 <sup>b</sup>	0.255 ± 0.008 <sup>a</sup>
d 14 to 28	0.555 ± 0.012 <sup>a</sup>	0.601 ± 0.012 <sup>b</sup>	0.598 ± 0.014 <sup>b</sup>
d 0 to 28	0.398 ± 0.008 <sup>a</sup>	0.433 ± 0.009 <sup>b</sup>	0.421 ± 0.008 <sup>b</sup>
ADFI, kg×pen <sup>-1</sup> ×d <sup>-1</sup>			
d 0 to 14	1.32 ± 0.03	1.34 ± 0.04	1.36 ± 0.04
d 14 to 28	3.25 ± 0.11	3.23 ± 0.10	3.18 ± 0.09
d 0 to 28	2.32 ± 0.06	2.26 ± 0.06	2.23 ± 0.06
G:F, kg/kg			
d 0 to 14	0.720 ± 0.029	0.801 ± 0.025	0.756 ± 0.028
d 14 to 28	0.675 ± 0.016 <sup>a</sup>	0.738 ± 0.015 <sup>b</sup>	0.745 ± 0.020 <sup>b</sup>
d 0 to 28	0.695 ± 0.019 <sup>a</sup>	0.756 ± 0.014 <sup>b</sup>	0.750 ± 0.021 <sup>b</sup>

<sup>a,b</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Values are least squares means ± SEM; for BW and ADG,  $n = 60$  to  $63$  per treatment; for ADFI and G:F,  $n = 16$  per treatment.

pigs consuming antibiotics or lysozyme, G:F was improved ( $P < 0.05$ ). Feed efficiency improved 9.8% from d 14 to 28 in pigs consuming antibiotics ( $P < 0.045$ ) or lysozyme ( $P = 0.042$ ) compared with pigs consuming the control diets. This resulted in an 8.3% improvement for the entire 28-d study for pigs consuming antibiotics or lysozyme compared with control diets ( $P = 0.048$ ).

Dietary treatment did not affect circulating IgA ( $P = 0.43$ ) or PUN ( $P = 0.51$ ) concentrations (Fig. 1). In addition, there were no sex differences in IgA ( $P = 0.69$ ) or PUN ( $P = 0.43$ ) concentrations. Circulating IgA increased from d 0 to 14 ( $P = 0.028$ ) and from d 14 to 28 ( $P < 0.022$ ) for all treatment groups. Plasma urea N increased from d 0 to 14, regardless of dietary treatment ( $P = 0.009$ ), and PUN concentrations at d 28 of treatment were similar to concentrations at d 14 ( $P = 0.65$ ).

No differences in TER were observed between dietary treatments (Fig. 2;  $P = 0.37$ ). However, gilts had a lower TER compared with barrows, regardless of dietary treatment ( $P = 0.035$ ).

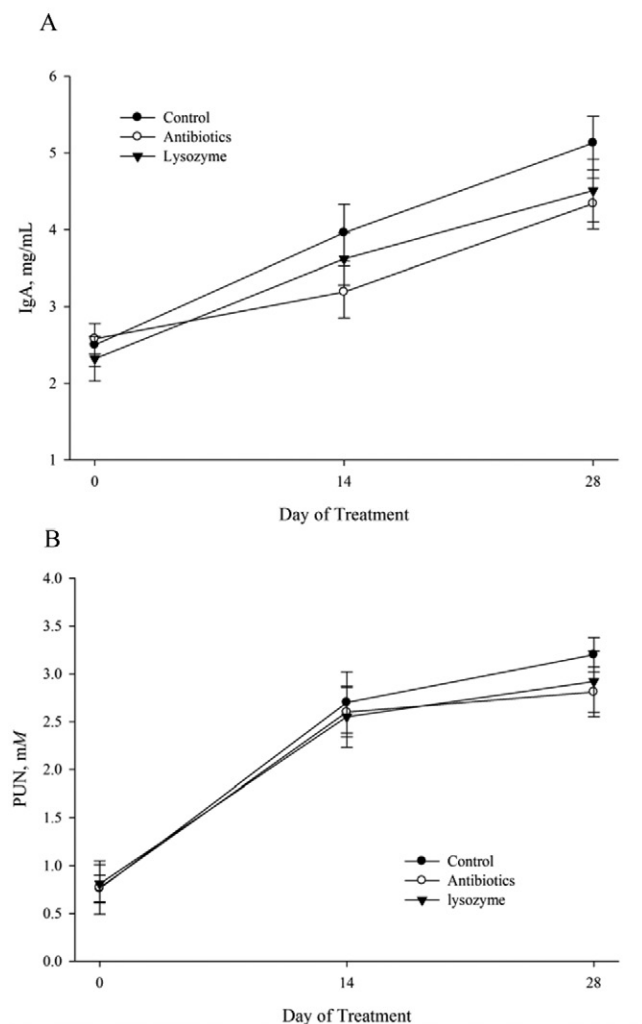
No morphological differences due to sex were observed ( $P = 0.66$ ). Neither villi height ( $P = 0.53$ ) nor crypt depth ( $P = 0.75$ ) differed in the ileum, regardless of dietary treatment (Fig. 3). However, villi height was 35% and 28% taller in the jejunum of pigs consuming antibiotics and lysozyme, respectively, compared with pigs consuming the control diet ( $P < 0.001$ ). In addition, crypt depth was 27% and 23% shorter in pigs consuming antibiotics and lysozyme, respectively, compared with pigs consuming the control diet ( $P < 0.001$ ). Because of the

morphological changes in the jejunum, the villi height to crypt depth ratio was increased 72% ( $P < 0.001$ ).

## DISCUSSION

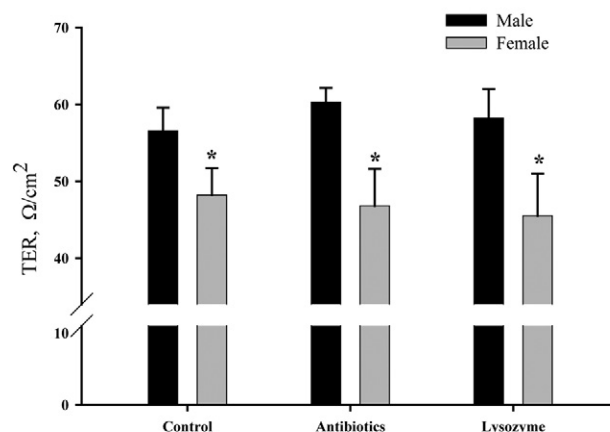
Antibiotics have been used as growth promoters for more than 50 yr, and the majority of swine produced in the United States receive subtherapeutic levels of antibiotics in their feed at some point during the production cycle. Antibiotic use has benefited producers by improving feed efficiency and decreasing the susceptibility to bacterial infections (Versteegen and Williams, 2002). In addition, subtherapeutic levels of antibiotics improve growth rates in several species, including swine (Schwarz et al., 2001; Cromwell, 2002; Thymann et al., 2007). In the current study, we showed that pigs weaned at 24 d of age consuming carbadox/copper sulfate or lysozyme in nursery diets had improved growth rates and feed efficiency. This is the first example of lysozyme improving feed efficiency in swine, where pigs consuming lysozyme had improved G:F of 8% compared with pigs consuming the control diet, which was similar to pigs consuming the antibiotic-treated feed. In our previous study (May et al., 2012), we did not observe differences in feed efficiency in 10-d-old pigs consuming lysozyme in liquid diets. However, our statistical power to detect pen differences in that experiment was extremely limited. Similar to the current experiment, Humphrey et al. (2002) observed increased feed efficiency in chicks consuming 153 mg human lysozyme/kg feed derived from transgenic rice. However, growth rate of chicks was not improved. The growth rate of pigs in the current study was increased approximately 7.3% for both antibiotic- and lysozyme-fed pigs, which is consistent with our previous study (May et al., 2012). Previous studies using human lysozyme from transgenic goat milk did not show an increase in growth rate of pigs consuming the transgenic goat milk (Maga et al., 2006; Brundige et al., 2008). As we postulated in our previous study, the lack of a growth response in these experiments is likely due to the use of antibiotics in addition to the human lysozyme. Because of the study design of both experiments (Maga et al., 2006; Brundige et al., 2008), it is not clear how much human lysozyme was actually consumed by the piglets. The amount of lysozyme was sufficient to alter intestinal morphology and microflora but may not have been adequate to improve growth performance. Data from the current experiment, as well as our previous efforts (May et al., 2012), clearly show that this source of lysozyme improves growth performance from 10 d through the nursery phase of production.

Given the improvements in growth rate in pigs consuming lysozyme or antibiotics, it is reasonable to expect that PUN may be altered as circulating PUN is a



**Figure 1.** Effect of antibiotics or lysozyme in nursery pig diets on circulating (A) IgA and (B) plasma urea nitrogen (PUN) in pigs weaned from the sow at 10 d of age. Values shown are means  $\pm$  SEM;  $n = 60$  to 63. No treatment differences were observed ( $P > 0.05$ ). The IgA increased from d 0 to 14 and from d 14 to 28 ( $P < 0.05$ ), and PUN increased from d 0 to 7 ( $P < 0.03$ ).

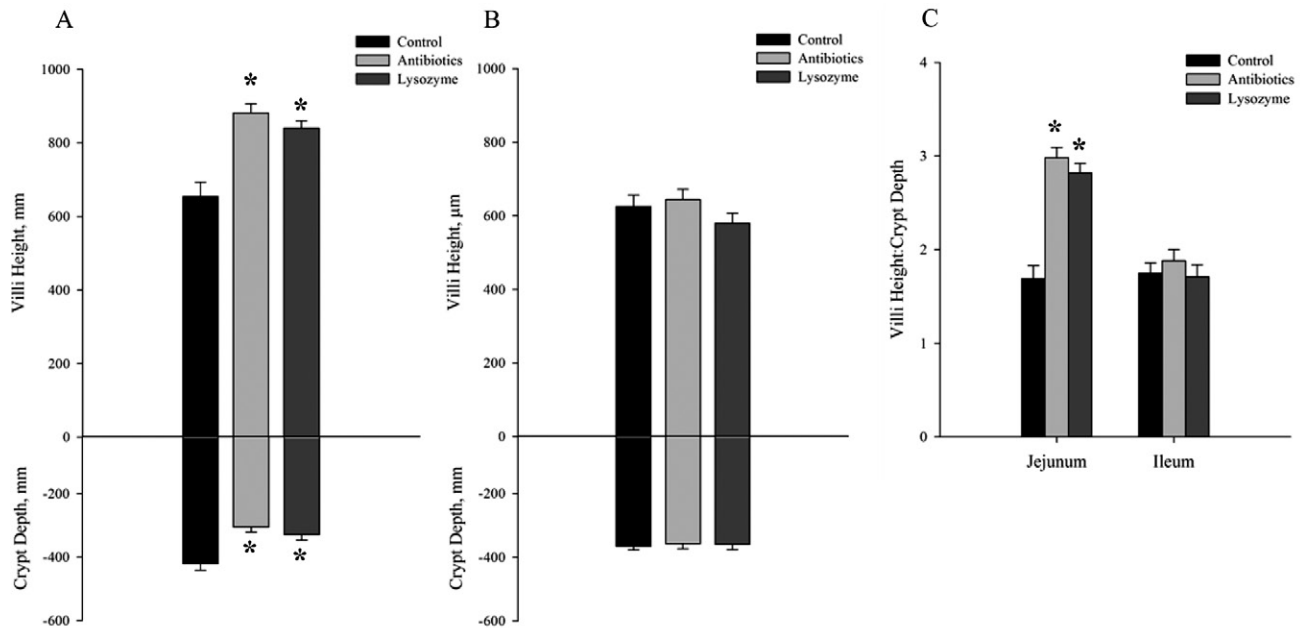
reliable indirect measurement to show the oxidation of dietary AA in young pigs. However, similar to our previous work (May et al., 2012), no treatment differences in PUN were observed in the current experiment, which suggests that pigs consuming lysozyme or antibiotics were using dietary AA for protein synthesis similarly to pigs consuming the nonmedicated control diets. The lack of observed differences in PUN is likely due, in part, to an excess of dietary AA. In addition, protein degradation increases as animals age (Goll et al., 2008), which contributes to circulating PUN. Plasma urea N increased from d 0 to 14 in the current experiment and then remained unchanged for the duration of the experiment. The postweaning increase in PUN is expected because, due to milk yield restraints, the sow limits milk intake of piglets by about 10 d of age (Boyd et al., 1995; Azain et al., 1996), which leads to low PUN concentrations at weaning. As the animals consume feed ad libitum, catabolism of excess AA increases PUN concentrations.



**Figure 2.** Effect of antibiotics or lysozyme in nursery pig diets on transepithelial electrical resistance (TER) of the jejunum. Values shown are means  $\pm$  SEM;  $n = 6$ . \*Mean differs from males consuming the same diet ( $P < 0.05$ ). For all other comparisons, there is no difference ( $P > 0.05$ ).

Immunoglobulin A is produced in the gastrointestinal tract in response to bacterial interactions (Suzuki and Fagarasan, 2008), and spray-dried plasma proteins, which have antimicrobial-like properties, reduce circulating IgA in pigs challenged with *Escherichia coli* K88 (Van den Broeck et al., 1999; Bosi et al., 2004). Thus, circulating IgA may be an indicator of the immunomodulatory effects of lysozyme and/or antibiotics. However, IgA did not differ in the current experiment, which indicates consumption of antibiotics or lysozyme did not alter the production of IgA. This agrees with our previous work with lysozyme in younger pigs (May et al., 2012) but not with work from our laboratories feeding antibiotics (chlorotetracycline/bacitracin) in grow-finish pigs (Wells et al., 2013). Circulating IgA was decreased after 8 wk (approximately 84 kg BW) of treatment in pigs consuming antibiotics compared with pigs consuming a nonmedicated control diet. The differences in IgA observed by Wells et al. (2013) may be due to the differing ages of the pigs in the current study, as well as the different antibiotics administered.

Changes in intestinal morphology due to antibiotic treatment are variable; some studies show improvements (Piva et al., 2008; May et al., 2012), whereas others do not (Thymann et al., 2007; Shen et al., 2009). Similar to our previous work (May et al., 2012), pigs in the current study had improved villus heights and crypt depths, which indicates improved intestinal health (Argenzio et al., 1990; Zijlstra et al., 1996; Oliver et al., 2002). However, the major morphological responses in pigs consuming lysozyme in liquid diets have been observed in the ileum (May et al., 2012) compared with responses seen exclusively in the jejunum in the current experiment. It is likely that the differences are due to the different physical forms of the diets consumed. Major



**Figure 3.** Effect of antibiotics or lysozyme in nursery pig diets on mean villus heights and crypt depths in the (A) jejunum and (B) ileum of pigs weaned from the sow. (C) The ratio of villi height to crypt depth. Values shown are means  $\pm$  SEM;  $n = 48$  to  $51$ . \*Mean differs from control ( $P < 0.001$ ). For all other comparisons, there is no difference ( $P > 0.05$ ).

changes occur in the gastrointestinal tract in response to the transition from a liquid to a dry diet (Koldovsky et al., 1995), in particular to ion transport (Pacha, 1997). Presumably, the changes in structure and function of the small intestine allowed lysozyme and antibiotics to have a greater effect on the jejunum in the current study. In the current experiment, crypt depths were decreased in pigs consuming lysozyme or antibiotics, whereas they were increased in pigs consuming lysozyme in liquid diets (May et al., 2012). This is likely because cellular proliferation is very high in the crypts in the younger animal, whereas villi enterocytes are longer-lived in suckling animals compared with weaned animals (Smith, 1988). It is likely crypt cells were proliferating as they were not migrated up the villi to replace sloughed enterocytes and that lysozyme and antibiotics simply improved this important function of the crypts.

Previous work with human lysozyme from transgenic goat milk or transgenic rice did not show improvements in intestinal morphology in the jejunum or ileum (Humphrey et al., 2002; Brundige et al., 2008; Cooper et al., 2011). Again, this is likely due to the concomitant presence of antibiotics in the consumed feed or simply a decreased consumption of lysozyme. However, Nyachoti et al. (2012) observed increased villi height in the ileum of pigs weaned at 17 d and fed the same source of lysozyme as the current study. Unfortunately, jejunum morphology was not measured. Changes in ileum morphology, unlike in the current study, are likely due to the effect of the *Escherichia coli* K88 challenge on the small intestine (Nyachoti et al., 2012). Nonetheless, taken together, the current study and previous work (May et al., 2012; Nyachoti et al., 2012)

indicate that this source of lysozyme improves small-intestinal morphology. Improvements in small-intestinal morphology may lead to a greater absorptive capacity and may be a mechanism by which lysozyme and antibiotics improve growth rates.

In addition to morphology measurements, jejunum TER was measured as an indicator of intestinal health. Transepithelial electrical resistance is a measurement of barrier function of an epithelial layer (Argenzio and Liacos, 1990; Moeser et al., 2007). There were no differences in TER between pigs consuming control, antibiotics, or lysozyme diets in the current study, indicating neither antibiotics nor lysozyme affect the barrier function of the jejunum. To our knowledge, this is the first assessment of TER on pigs consuming antimicrobials vs. nonmedicated feed. Interestingly, gilts had a decreased TER compared with barrows, regardless of dietary treatment. Although we have no definitive explanation for the gender effects, gender differences have been observed in ion flux experiments in the murine model (Homma et al., 2005; O'Mahony et al., 2007; Al-Nakkash et al., 2011), and it appears that differences are mediated by estradiol. Although gilts in the current experiment were likely prepubertal, sex steroids, or the lack thereof, may be mediating differences in TER.

Subtherapeutic antibiotics continue to be an efficacious growth promoter in swine diets. However, the perceived danger of their use, and their potential to be regulated out of use, compels the search for effective alternatives. This study shows that lysozyme improves growth rate when fed in dry nursery diets. In addition, to the knowledge of the authors, this study is the first to

demonstrate improved feed efficiency in response to lysozyme consumption in pigs. Small-intestinal morphology was also improved in nursery pigs consuming lysozyme. Whether intestinal changes are mechanistically responsible for improved feed conversion remains to be seen. Thus, we concluded that lysozyme is a suitable alternative to carbadox/copper sulfate diets fed to pigs weaned from the sow at 24 d of age.

## LITERATURE CITED

- Al-Nakkash, L., L. Batia, M. Bhakta, A. Peterson, N. Hale, R. Skinner, S. Sears, and J. Jensen. 2011. Stimulation of murine intestinal secretion by daily genistein injections: Gender-dependent differences. *Cell. Physiol. Biochem.* 28:239–250.
- Argenzio, R. A., and J. A. Liacos. 1990. Endogenous prostanoids control ion transport across neonatal porcine ileum in vitro. *Am. J. Vet. Res.* 51:747–751.
- Argenzio, R. A., J. A. Liacos, M. L. Levy, D. J. Meuten, J. G. Lecce, and D. W. Powell. 1990. Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-Na absorption in enteric cryptosporidiosis of pigs. *Gastroenterology* 98:1129–1140.
- Azain, M. J., T. Tomkins, J. S. Sowinski, R. A. Arentson, and D. E. Jewell. 1996. Effect of supplemental pig milk replacer on litter performance: Seasonal variation in response. *J. Anim. Sci.* 74:2195–2202.
- Bosi, P., L. Casini, A. Finamore, C. Cremokolini, G. Merialdi, P. Trevisi, F. Nobili, and E. Mengheri. 2004. Spray-dried plasma improves growth performance and reduces inflammatory status of weaned pigs challenged with enterotoxigenic *Escherichia coli* K88. *J. Anim. Sci.* 82:1764–1772.
- Boyd, R. D., R. S. Kensinger, R. J. Harrell, and D. E. Bauman. 1995. Nutrient uptake and endocrine regulation of milk synthesis by mammary tissue of lactating sows. *J. Anim. Sci.* 73(Suppl. 2):36–56.
- Brundige, D. R., E. A. Maga, K. C. Klasing, and J. D. Murray. 2008. Lysozyme transgenic goats' milk influences gastrointestinal morphology in young pigs. *J. Nutr.* 138:921–926.
- Brundige, D. R., E. A. Maga, K. C. Klasing, and J. D. Murray. 2010. Consumption of pasteurized human lysozyme transgenic goats' milk alters serum metabolite profile in young pigs. *Transgenic Res.* 19:563–574.
- Clarke, T. B., K. M. Davis, E. S. Lysenko, A. Y. Zhou, Y. Yu, and J. N. Weiser. 2010. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat. Med.* 16:228–231.
- Cooper, C. A., D. R. Brundige, W. A. Reh, E. A. Maga, and J. D. Murray. 2011. Lysozyme transgenic goats' milk positively impacts intestinal cytokine expression and morphology. *Transgenic Res.* 20:1235–1243.
- Cromwell, G. L. 2002. Why and how antibiotics are used in swine production. *Anim. Biotechnol.* 13:7–27.
- Ellison, R. T., III and T. J. Giehl. 1991. Killing of gram-negative bacteria by lactoferrin and lysozyme. *J. Clin. Invest.* 88:1080–1091.
- Goll, D. E., G. Neti, S. W. Mares, and V. F. Thompson. 2008. Myofibrillar protein turnover: The proteasome and the calpains. *J. Anim. Sci.* 86:E19–E35.
- Homma, H., E. Hoy, D. Z. Xu, Q. Lu, R. Feinman, and E. A. Deitch. 2005. The female intestine is more resistant than the male intestine to gut injury and inflammation when subjected to conditions associated with shock states. *Am. J. Physiol. Gastrointest. Liver Physiol.* 288:G466–G472.
- Humphrey, B. D., N. Huang, and K. C. Klasing. 2002. Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. *J. Nutr.* 132:1214–1218.
- Kawano, M., Y. Namba, and M. Hanaoka. 1981. Regulatory factors of lymphocyte-lymphocyte interaction. I. Con A-induced mitogenic factor acts on the late G1 stage of T-cell proliferation. *Microbiol. Immunol.* 25:505–515.
- Koldovsky, O., M. Dobiasova, P. Hahn, J. Kolinska, J. Kraml, and J. Pacha. 1995. Development of gastrointestinal functions. *Physiol. Res.* 44:341–348.
- Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill, New York.
- Maga, E. A., R. L. Walker, G. B. Anderson, and J. D. Murray. 2006. Consumption of milk from transgenic goats expressing human lysozyme in the mammary gland results in the modulation of intestinal microflora. *Transgenic Res.* 15:515–519.
- May, K. D., J. E. Wells, C. V. Maxwell, and W. T. Oliver. 2012. Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs. *J. Anim. Sci.* 90:1118–1125.
- Moeser, A. J., K. A. Ryan, P. K. Nighot, and A. T. Blikslager. 2007. Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 293:G413–G421.
- NRC. 1998. Nutrient requirements of swine. 10th ed. Natl. Acad. Press, Washington, DC.
- Nyachoti, C. M., E. Kiarie, S. K. Bhandari, G. Zhang, and D. O. Krause. 2012. Weaned pig responses to *Escherichia coli* K88 (ETEC) oral challenge when receiving a lysozyme-supplement. *J. Anim. Sci.* 90:252–260.
- O'Mahony, F., R. Alzamora, V. Betts, F. LaPaix, D. Carter, M. Irnaten, and B. J. Harvey. 2007. Female gender-specific inhibition of KCNQ1 channels and chloride secretion by 17 $\beta$ -estradiol in rat distal colonic crypts. *J. Biol. Chem.* 282:24563–24573.
- Oliver, W. T., S. A. Mathews, O. Phillips, E. E. Jones, J. Odle, and R. J. Harrell. 2002. Efficacy of partially hydrolyzed corn syrup solids as a replacement for lactose in manufactured liquid diets for neonatal pigs. *J. Anim. Sci.* 80:143–153.
- Oliver, W. T., and J. R. Miles. 2010. A low-fat liquid diet increases protein accretion and alters cellular signaling for protein synthesis in 10-day-old pigs. *J. Anim. Sci.* 88:2576–2584.
- Oliver, W. T., J. R. Miles, D. E. Diaz, J. J. Dibner, G. E. Rottinghaus, and R. J. Harrell. 2012. Zearalenone enhances reproductive tract development, but does not alter skeletal muscle signaling in prepubertal gilts. *Anim. Feed. Sci. Technol.* 174:79–85.
- Pacha, J. 1997. Ontogeny of Na<sup>+</sup> transport in rat colon. *Comp. Biochem. Physiol. A Physiol.* 118:209–210.
- Piva, A., E. Grilli, L. Fabbri, V. Pizzamiglio, P. P. Gatta, F. Galvano, M. Bognanno, L. Fiorentini, J. Wolinski, R. Zabielski, and J. A. Patterson. 2008. Intestinal metabolism of weaned piglets fed a typical United States or European diet with or without supplementation of tributyrin and lactitol. *J. Anim. Sci.* 86:2952–2961.
- Schwarz, S., C. Kehrenberg, and T. R. Walsh. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *Int. J. Antimicrob. Agents* 17:431–437.
- Shen, Y. B., X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effects of yeast culture and supplementation on growth performance, intestinal health, and immune response of nursery pigs. *J. Anim. Sci.* 87:2614–2624.
- Silhavy, T. J., D. Kahne, and S. Walker. 2010. The bacterial cell envelope. *Cold Spring Harb. Perspect. Biol.* 2:A000414.
- Smith, M. W. 1988. Postnatal development of transport function in the pig intestine. *Comp. Biochem. Physiol. A Comp. Physiol.* 90:577–582.
- Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and procedures of statistics: A biomedical approach. 3rd ed. McGraw-Hill, New York.

- Suzuki, K., and S. Fagarasan. 2008. How host-bacterial interactions lead to IgA synthesis in the gut. *Trends Immunol.* 29:523–531.
- Thymann, T., K. U. Sorensen, M. S. Hedemann, J. Elnif, B. B. Jensen, H. Banga-Mboko, T. D. Leser, and P. T. Sangild. 2007. Antimicrobial treatment reduces intestinal microflora and improves protein digestive capacity without changes in villous structure in weanling pigs. *Br. J. Nutr.* 97:1128–1137.
- Van den Broeck, W., E. Cox, and B. M. Goddeeris. 1999. Receptor-dependent immune responses in pigs after oral immunization with F4 fimbriae. *Infect. Immun.* 67:520–526.
- Verstegen, M. W., and B. A. Williams. 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. *Anim. Biotechnol.* 13:113–127.
- Wells, J. E., N. Kalchayanand, E. D. Berry, and W. T. Oliver. 2013. Effects of antimicrobials fed as dietary growth promoters on faecal shedding of *Campylobacter*, *Salmonella*, and shiga-toxin producing *Escherichia coli* in swine. *J. Appl. Microbiol.* 114(2):318–328.
- Zijlstra, R. T., K. Y. Whang, R. A. Easter, and J. Odle. 1996. Effect of feeding a milk replacer to early-weaned pigs on growth, body composition, and small intestinal morphology, compared with suckled littermates. *J. Anim. Sci.* 74:2948–2959.